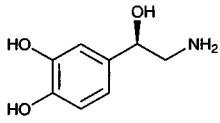
HPLC VARIABLES
Column: 250 × 4.6 10 μm R-SIL-Amine (NH2) (Alltech) (Change 2 μm filter in front of column before each run.)
Mobile phase: MeCN:THF 2:98
Flow rate: 1
Injection volume: 20
Detector: F ex 227 em 612

CHROMATOGRAM
Retention time: 5.7
Internal standard: 4-octylphenol (4.0)
Limit of detection: 480 ng/mL
Limit of quantitation: 3.125 μg/mL

REFERENCE
McPherson,J.L.; Nichols,J.H.; Barditch-Crovo,P.; Hamzeh,F.M. Determination of the spermicide nonoxynol-9 in vaginal lavage by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *677*, 204–208.

Norepinephrine



Molecular formula: $C_8H_{11}NO_3$
Molecular weight: 169.18
CAS Registry No.: 51-41-2, 69815-49-2 (bitartrate monohydrate), 51-40-1 (bitartrate)
Merck Index: 6788

SAMPLE
Matrix: blood
Sample preparation: 20 mL Whole blood + 1 mL 20 mg/mL EDTA solution containing 10 mg/mL sodium metabisulfite, mix, centrifuge at 4° at 4000 g for 10 min. Remove the plasma and add concentrated perchloric acid until the concentration of perchloric acid is 400 mM, mix, let stand in the cold for 15 min, centrifuge at 4° at 20000 g for 20 min. Adjust pH of 2 mL supernatant to 7.0 ± 0.2 with 500 mM KOH, add 400 μL 875 μg/mL o-phthalaldehyde in pH 10.40 ± 0.02 buffer (containing 2-mercaptoethanol ?), add 2 g NaCl, add 2 mL ethyl acetate, shake for 1 min, centrifuge at 3400 g, repeat the extraction. Combine the organic layers and add them to 2 mL 35 mM pH 10.0 ± 0.1 Na_2HPO_4 buffer, shake for 1 min, centrifuge at 3400 g, discard the aqueous layer, wash the ethyl acetate layer again with phosphate buffer. Reduce the ethyl acetate volume to 100 μL under a stream of nitrogen, inject a 10-50 μL aliquot.

HPLC VARIABLES
Guard column: Co:Pell ODS
Column: 300 × 4 10 μm μBondapak phenyl
Mobile phase: Gradient. MeCN:25 mM pH 5.10 NaH_2PO_4 buffer 25:75 for 15 min then MeOH:25 mM pH 5.10 NaH_2PO_4 buffer 45:55 (step gradient).
Column temperature: 26
Flow rate: 1.5
Injection volume: 10-50
Detector: F ex 340 em 480

CHROMATOGRAM
Retention time: 11
Internal standard: tyramine (44)
Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: dopamine, serotonin

KEY WORDS
plasma; whole blood; pig; derivatization

REFERENCE
Davis,T.P.; Gehrke,C.W.,Jr.; Williams,C.H.; Gehrke,C.W.; Gerhardt,K.O. Pre-column derivatization and high-performance liquid chromatography of biogenic amines in blood of normal and malignant hyperthermic pigs, *J.Chromatogr.*, **1982**, *228*, 113–122.

SAMPLE
Matrix: blood
Sample preparation: Chill 20 mL whole blood in ice water, add 1 mL reagent, invert several time, centrifuge at 4° at 4000 g for 10 min. Remove the plasma and make it 0.4 M in perchloric acid by adding concentrated perchloric acid, mix, let stand at 4° for 15 min, centrifuge at 4° at 20000 g for 20 min. Remove a 2 mL aliquot and adjust the pH to 7.0 ± 0.2 with 0.5 M KOH, add 400 μL reagent, add 2 g NaCL, add 2 mL ethyl acetate, shake for 1 min, centrifuge at 3400 g, repeat the extraction. Combine the organic layers, add 2 mL 35 mM pH 10.0 ± 0.1 Na_2HPO_4 buffer, shake for 1 min, centrifuge at 3400 g, repeat the wash, evaporate the ethyl acetate layer to 100 μL with a stream of nitrogen, inject a 10-50 μL aliquot. (Reagent was 875 μg/mL o-phthalaldehyde and 2-mercaptoethanol in pH 10.40 ± 0.2 potassium borate buffer.)

HPLC VARIABLES
Guard column: Co:Pell ODS
Column: 300 × 4 10 μm μBondapak phenyl
Mobile phase: Gradient. MeCN:25 mM pH 5.10 NaH_2PO_4 25:75 for 15 min, then MeOH:25 mM pH 5.10 NaH_2PO_4 45:55 for 35 min (step gradient).
Column temperature: 26
Flow rate: 1.5
Injection volume: 10-50
Detector: F ex 340 em 480

CHROMATOGRAM
Retention time: 12
Limit of detection: <0.5 ng/mL

OTHER SUBSTANCES
Extracted: dopamine, histamine, octopamine, serotonin, tyramine

KEY WORDS
pig; whole blood; derivatization

REFERENCE
Davis,T.P.; Gehrke,C.W.,Jr.; Williams,C.H.; Gehrke,C.W.; Gerhardt,K.O. Pre-column derivatization and high-performance liquid chromatography of biogenic amines in blood of normal and malignant hyperthermic pigs, *J.Chromatogr.*, **1982**, *228*, 113–122.

SAMPLE
Matrix: blood
Sample preparation: Plasma. Prepare a SPE column by adding 500 μL of a 20% suspension of 19-40 μm Toyopak SP (strong cation-exchange sulfopropyl resin, Na^+ (Toyo Soda)) in water to a 35 × 6 column, wash with two 1 mL portions of 2 M LiOH, wash with two 5 mL portions of water, wash with two 1 mL portions of EtOH:12 M HCl 90:10, wash with two 5 mL portions of water, wash with three 1 mL portions of buffer. 500 μL Plasma + 25 μL 10 nM isoproterenol + 500 μL buffer, mix, add to the SPE column, wash with two 5 mL portions of water, wash with 1 mL MeCN:water 50:50, elute with 300 μL 600 μM potassium ferricyanide in 600 mM KCl:MeCN 50:50, add 50 μL reagent to the eluate, heat at 37° for 40 min, cool in ice-water, inject a 100 μL aliquot. Urine. 10 μL Urine + 1 mL MeCN:500 mM KCl 60:40 + 10 μL 500 nM isoproterenol + 10 μL 75 mM potassium hexacyanoferrate(III) + 100 μL reagent, heat at 37° for 40 min, inject a 100 μL aliquot (J. Chromatogr. 1986, 380, 229). (Prepare buffer by mixing 8 volumes 250 mM LiOH in 200 mM phosphoric acid with 1 volume 200 mM phosphoric acid, pH 5.8. Prepare reagent by dissolving 212 mg 1,2-diphenylethylenediamine in 10 mL 100 mM HCl, pH 6.7.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm TSK-gel ODS-120T (Toyo Soda)
Mobile phase: MeCN:MeOH:50 mM pH 7.0 Tris-HCl buffer 50:10:40 (Wash with MeCN:MeOH: water 50:10:40 for 15 min at the end of each day.)
Flow rate: 1
Injection volume: 100
Detector: F ex 345 em 485 (plasma), F ex 350 em 480 (urine)

CHROMATOGRAM
Retention time: 3
Internal standard: isoproterenol (8)
Limit of detection: 7 pM

OTHER SUBSTANCES
Extracted: dopamine, epinephrine

KEY WORDS
derivatization; plasma; SPE

REFERENCE
Mitsui,A.; Nohta,H.; Ohkura,Y. High-performance liquid chromatography of plasma catecholamines using 1,2-diphenylethylenediamine as precolumn fluorescence derivatization reagent, *J.Chromatogr.*, **1985**, *344*, 61–70.

SAMPLE
Matrix: blood
Sample preparation: Prepare a 20 × 5 polypropylene column packed with CM-Sephadex preswollen in water, wash with 5 mL 2 M HCl, wash with 10 mL water, wash with 10 mL 100 mM pH 7 phosphate buffer. 1 mL Plasma + 30 μL 80 ng/mL N-methyldopamine, apply to column, wash with 5.5 mL water (A), elute with 3 mL 0.5 M perchloric acid. Collect eluate, add 2 mL 1.5 M pH 9.3 Tris buffer containing 60 mM EDTA, add 20 mg alumina, vortex for 2 min, discard supernatant, add 2 mL water to alumina, mix, centrifuge at 3000 g for 3 min, repeat water wash, remove as much water as possible, elute catecholamines from alumina with 100 μL 100 mM acetic acid with vortexing for 2 min, centrifuge, inject 25 μL aliquot of supernatant. (Wash water A contains levodopa, carbidopa, DOPAC, and O-methyldopa.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil C18
Mobile phase: MeCN:MeOH:25 mM sodium acetate 4:4:92 containing 0.2 mM 1-octanesulfonic acid and 0.3 mM disodium EDTA, pH was adjusted to pH 3 with acetic acid
Flow rate: 0.9
Injection volume: 10
Detector: E, ESA Coulochem 5100 A, 5010 A analytical cell, first electrode +0.25 V, second electrode -0.30 V

CHROMATOGRAM
Retention time: 8
Internal standard: N-methyldopamine (16)
Limit of detection: 0.5 ng/mL

OTHER SUBSTANCES
Simultaneous: epinephrine, dopamine

KEY WORDS
plasma; SPE

REFERENCE
Betto,P.; Ricciarello,G.; Giambenedetti,M.; Lucarelli,C.; Ruggeri,S.; Stocchi,F. Improved high-performance liquid chromatographic analysis with double detection system for L-dopa, its metabolites and carbidopa in plasma of parkinsonian patients under L-dopa therapy, *J.Chromatogr.*, **1988**, *459*, 341–349.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 250 μL 1 ng/mL α-methylnorepinephrine + 1 mL buffer + 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 20° at 1000 g for 5 min, freeze in acetone/dry ice. Remove the organic phase and add it to 2 mL 1-octanol and 200 μL 80 mM acetic acid, shake, centrifuge at 20° at 1000 g for 5 min, freeze in acetone/dry ice. Discard the organic phase, thaw the aqueous phase and add it to 1 mL 10 mM HCl, 1 mL buffer, and 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 20° at 1000 g for 5 min, freeze in acetone/dry ice. Remove the organic phase and add it to 2 mL 2 M pH 8.6 ammonia/ammonium chloride buffer containing 13.4 mM EDTA, shake, freeze in dry ice/acetone. Remove the organic layer and add it to 2 mL 1-octanol and 150 μL 80 mM acetic acid, shake, centrifuge at 20° at 1000 g for 5 min, freeze in dry ice/acetone, discard the organic layer. Thaw the aqueous layer and add it to 250 μL MeCN, 50 μL 1.75 M pH 7.05 bicine, and 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, add 20 μL 20 mM potassium ferricyanide in water, heat at 37° in the dark for 1 h, keep at 20° in the dark, inject a 100 μL aliquot. (Buffer was 2 M pH 8.6 ammonia/ammonium chloride buffer containing 8.9 mM diphenylborate-ethanolamine complex and 13.4 mM EDTA. Stir buffer with 45 g/L activated alumina for 2 h before use. Wash 1-octanol with 80 mM acetic acid. Recrystallize 1,2-diphenylethylenediamine from toluene:light petroleum (bp 60-80°) 10:90, dry overnight at 60°.)

HPLC VARIABLES
Column: 100 × 4.6 3 μm Cp MicroSpher C18 (Chrompack)
Mobile phase: MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 40:8:50
Flow rate: 1
Injection volume: 100
Detector: F ex 350 em 480

CHROMATOGRAM
Retention time: 2
Internal standard: α-methylnorepinephrine (3)
Limit of detection: 2 pg/mL

OTHER SUBSTANCES
Extracted: dihydroxybenzylamine, dopamine, epinephrine, isoproterenol

KEY WORDS
plasma; derivatization; comparison with electrochemical detection

REFERENCE
van der Hoorn,F.A.J.; Boomsma,F.; Man in 't Veld,A.J.; Schalekamp,M.A.D.H. Determination of catecholamines in human plasma by high-performance liquid chromatography: comparison between a new method with fluorescence detection and an established method with electrochemical detection, *J.Chromatogr.*, **1989**, *487*, 17–28.

SAMPLE
Matrix: blood
Sample preparation: Pack a 65 × 15 SPE column with 50 mg WA-4 alumina (Sigma). Add 500 μL plasma to the SPE column, add 1 mL buffer, rotate for 15 min, wash three times with water (aspirating to dryness each time), centrifuge to dryness, add 200 μL 100 mM pH 1.2 perchloric acid, mix, let stand for 15 min, centrifuge the SPE column at 1000 g for 3 min, inject an aliquot of the effluent. (Buffer was 45 g Tris and 5 g EDTA in 200 mL water, pH adjusted to 8.6 with concentrated HCl.)

HPLC VARIABLES
Guard column: 50 × 4.6 5 μm reversed-phase
Column: 250 × 4.6 5 μm ODS Spherisorb
Mobile phase: Buffer contained 1.4% monochloroacetic acid, 0.47% NaOH, and 0.075% EDTA, finally pH adjusted to 3.0 with NaOH or monochloroacetic acid and 6 mg% sodium octylsulfate added.
Column temperature: 35
Flow rate: 1

Injection volume: 100
Detector: E, Bioanalytical Systems LC-4B, TL-5 transducer with a glassy carbon electrode, +650 mV, 1 nA, Ag/AgCl reference electrode

OTHER SUBSTANCES
Extracted: epinephrine, dopamine

KEY WORDS
plasma; rabbit; human; SPE

REFERENCE
Ganhao,M.F.; Hattingh,J.; Hurwitz,M.L.; Pitts,N.I. Evaluation of a simple plasma catecholamine extraction procedure prior to high-performance liquid chromatography and electrochemical detection, *J.Chromatogr.*, **1991**, *564*, 55–66.

SAMPLE
Matrix: blood
Sample preparation: Plasma. 1 mL Plasma + 125 μL 2 ng/mL α-methylnorepinephrine + 1 mL buffer + 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 1000 g for 5 min, freeze in dry ice/acetone. Remove the organic phase and add it to 2 mL 1-octanol (saturated with 80 mM acetic acid) and 200 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze the aqueous layer and remove the organic layer. Add 1 mL 10 mM HCl, 1 mL buffer, and 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol to the aqueous phase. Shake, centrifuge, freeze, remove the organic layer and add it to 2 mL 2 M pH 8.6 ammonia-ammonium chloride buffer containing 13.4 mM EDTA (but no complex). Freeze, remove the organic layer and add it to 2 mL 1-octanol and 150 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze, remove the organic layer and add the aqueous layer to 200 μL MeCN, 50 μL 1.75 M pH 6.95 bicine buffer containing 1% EDTA, 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, and 20 μL 20 mM potassium ferricyanide in water. Heat at 37° in the dark for 1 h, inject a 75 μL aliquot (keep it in the dark in the autosampler). Urine. 100 μL Urine + 1 mL 10 mM HCl + 125 μL 40 ng/mL α-methylnorepinephrine + 1 mL buffer + 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol, shake for 2 min, centrifuge at 1000 g for 5 min, freeze in dry ice/acetone. Remove the organic phase and add it to 2 mL 1-octanol (saturated with 80 mM acetic acid) and 200 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze the aqueous layer and remove the organic layer. Add 1 mL 10 mM HCl, 1 mL buffer, and 5 mL n-heptane containing 4.6 mM tetraoctylammonium bromide and 10 mL/L 1-octanol to the aqueous phase. Shake, centrifuge, freeze, remove the organic layer and add it to 2 mL 1-octanol and 150 μL 80 mM acetic acid, shake, centrifuge at 1000 g for 5 min. Freeze, remove the organic layer and add the aqueous layer to 200 μL MeCN, 50 μL 1.75 M pH 6.95 bicine buffer containing 1% EDTA, 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, and 20 μL 20 mM potassium ferricyanide in water. Heat at 37° in the dark for 1 h, inject a 50 μL aliquot (keep it in the dark in the autosampler). (Buffer was a 2 M pH 8.6 ammonia-ammonium chloride buffer containing 8.9 mM diphenyl borate-ethanolamine complex and 13.4 mM EDTA.)

HPLC VARIABLES
Column: 100 × 4.6 3 μm PhaseSep C18 ODS2
Mobile phase: Gradient. A was MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 20:4:76. B was MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 60:10:30. A:B 40:60 for 3 min, go to 0:100 over 0.5 min, stay at 0:100 for another 4.5 min. (After the last sample flush column with 60 mL MeCN:MeOH:water 70:10:20.)
Flow rate: 1
Injection volume: 50-75
Detector: F ex 350 em 480

CHROMATOGRAM
Retention time: 2
Internal standard: α-methylnorepinephrine (2.5)
Limit of detection: 0.3-0.6 pg

OTHER SUBSTANCES
Simultaneous: epinephrine, dopamine, epinine

Interfering: α-methyldopa

KEY WORDS
plasma; derivatization

REFERENCE
Boomsma,F.; Alberts,G.; van der Hoorn,F.A.J.; Man in 't Veld,A.J.; Schalekamp,M.A.D.H. Simultaneous determination of free catecholamines and epinine and estimation of total epinine and dopamine in plasma and urine by high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1992**, *574*, 109–117.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 5 mg alumina + 10 μL 10 μM IS in 10 mM perchloric acid + 100 μL pH 8.7 Tris-HCl buffer, stir for 10 min, centrifuge at 3000 g for 1 min, discard the supernatant. Wash the alumina with two 500 μL portions of water, add 100 μL 100 mM perchloric acid, mix for 1 min, centrifuge at 3000 g for 1 min, inject a 50 μL aliquot of the supernatant. (Heat 20 g alumina (WA-4, Sigma) with 200 mL 2 M HCl at 100° for 1 h with gentle mixing, decant the supernatant, wash with twenty 200 mL portions of water, filter (Toyo Roshi No. 2 paper), dry at 120° overnight.)

HPLC VARIABLES
Column: 150 × 4.6 catecholpak (JASCO)
Mobile phase: MeCN:50 mM pH 3.20 potassium acetate:50 mM pH 3.20 potassium phosphate buffer 3:92.15:4.85 containing 1 mM sodium hexanesulfonate
Column temperature: 40
Flow rate: 0.5
Injection volume: 50
Detector: Chemiluminescence (Kenko filter Y-46) following post-column reaction. The column effluent mixed with reagent 1 pumped at 0.25 mL/min and the mixture flowed through a 15 m × 0.5 mm i.d. knitted PTFE coil at 80°. The effluent from the coil mixed with reagent 2 pumped at 1.4 mL/min and this mixture flowed to the detector. (Reagent 1 was 105 mM ethylenediamine (semiconductor grade) and 175 mM imidazole in MeCN:EtOH 90:10. Reagent 2 was 0.25 mM bis[4-nitro-2-(3,6,9-trioxadecyloxycarbonyl)phenyl] oxalate (Wako), 150 mM hydrogen peroxide, and 110 mM trifluoroacetic acid in dioxane:ethyl acetate 50:50 (Caution! Dioxane is a carcinogen!).)

CHROMATOGRAM
Retention time: 14
Internal standard: 3,4-dihydroxybenzylamine (17)
Limit of detection: 1 fmole

OTHER SUBSTANCES
Extracted: dopamine, epinephrine

KEY WORDS
human; rat; plasma; SPE; post-column reaction

REFERENCE
Higashidate,S.; Imai,K. Determination of femtomole concentrations of catecholamines by high-performance liquid chromatography with peroxyoxalate chemiluminescence detection, *Analyst*, **1992**, *117*, 1863–1868.

SAMPLE
Matrix: blood
Sample preparation: Filter (Ultrafree-MC with 10000 molecular mass cut-off, Millipore) 100 μL plasma while centrifuging at 15000 g for 15 min. Mix 50 μL ultrafiltrate and 10 μL 140 ng/mL 3-methoxytyramine in Ringer solution, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 150 × 1 5 μm Inertsil-2 ODS

Mobile phase: MeCN:THF:water 6:0.8:93.2 containing 0.48 g/L sodium 1-octanesulfonate, 2 g/L NaH_2PO_4, 8.82 g/L sodium citrate, 10 mg/L EDTA, and 1 mL/L diethylamine, pH adjusted to 3.2 with concentrated orthophosphoric acid.
Flow rate: 0.06
Injection volume: 5
Detector: E, Bioanalytical Systems BAS-4C, glassy carbon working electrodes, upstream +0.75 V, downstream +0.05 V (measuring electrode), Ag/AgCl reference electrode

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CHROMATOGRAM
Retention time: 2.5
Internal standard: 3-methoxytyramine (11)
Limit of detection: 0.2-0.5 pg

OTHER SUBSTANCES
Extracted: dopamine, epinephrine, 3,4-dihydroxyphenylacetic acid, serotonin, 5-hydroxyindoleacetic acid, homovanillic acid

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KEY WORDS
plasma; microbore; rat; ultrafiltrate

REFERENCE
Cheng,F.-C.; Yang,L.-L.; Kuo,J.-S.; Yang,M.C.M.; Yu,P.-C. Rapid assay of the monoamine content in small volumes of rat plasma, *J.Chromatogr.B*, **1994**, *653*, 9–16.

SAMPLE
Matrix: blood, food, peptides, plants, tissue
Sample preparation: Hydrolyze peptide with 6 M HCl containing 0.2% 3,3'-thiodipropionic acid at 110° for 24 h, evaporate to dryness, reconstitute with 50-200 μL 0.1% HCl containing 0.2% 3,3'-thiodipropionic acid. Homogenize (Ultra-Turrax) 0.1-1 g food, tissue, plant material, lyophilized plasma, or lyophilized tissue in 10 mL 250 nM IS in 100 mM HCl containing 0.2% 3,3'-thiodipropionic acid at 20000 rpm for 2 min, sonicate for ≤30 min, centrifuge at 5000 g for 20 min, discard fat layer, filter (Millipore ultrafiltration insert (MW cutoff 5000) prewashed with 200 μL 100 mM HCl containing 0.2% 3,3'-thiodipropionic acid) 3 mL supernatant while centrifuging at 3500 g for 1 h. Mix 20 μL deproteinized sample (or 10 μL peptide hydrolysate) with 180 μL buffer, vortex, add 200 μL reagent, mix, heat at 70° for 15 min with mixing at 1 min and 12 min, cool in an ice bath for 5 min, centrifuge at 10000 g for 10 s, add 400 μL diluent, mix thoroughly, centrifuge at 15000 g for 5 min, inject a 10 μL aliquot of the supernatant. (Prepare buffer by dissolving 630 mg sodium bicarbonate in 40 mL water, adjusting pH to 8.6 with NaOH, and making up to 50 mL with water. Prepare reagent by sonicating 40 mg dabsyl chloride in 10 mL acetone for 10 min, then filtering into brown vials and storing at -20°. Prepare diluent by mixing 50 mL MeCN, 25 mL EtOH, and 25 mL mobile phase A.)

HPLC VARIABLES
Guard column: present but not specified
Column: 150 × 3.9 4 μm Novapak C18
Mobile phase: Gradient. A was DMF:9 mM NaH_2PO_4 containing 0.16% triethylamine, adjusted to pH 6.55 with phosphoric acid. B was MeCN:water 80:20. A:B 92:8 for 2 min, to 80:20 over 5 min (Waters convex curve 5), to 65:35 over 28 min (Waters concave curve 7), to 50:50 over 10 min, to 0:100 over 21 min, maintain at 0:100 for 11 min, return to initial conditions over 0.5 min, re-equilibrate for 12.5 min.
Column temperature: 50
Flow rate: 1
Injection volume: 10
Detector: UV 436

CHROMATOGRAM
Retention time: 68.49
Internal standard: norleucine (40.90), norvaline (35.06)

OTHER SUBSTANCES
Extracted: amino acids dopamine, epinephrine, histamine, taurine

KEY WORDS
rinse glass and plasticware with 70% EtOH and water and dry before use; derivatization; cheese; meat; sausage; fish; plasma

REFERENCE
Krause,I.; Bockhardt,A.; Neckermann,H.; Henle,T.; Klostermeyer,H. Simultaneous determination of amino acids and biogenic amines by reversed-phase high-performance liquid chromatography of the dabsyl derivatives, *J.Chromatogr.A*, **1995**, *715*, 67–79.

SAMPLE
Matrix: blood, urine
Sample preparation: Serum. Add 1 mL serum to 100 mg activated aluminum oxide suspended in 1 mL pH 8.7 Tris-HCl buffer, stir, let stand for 10 min. Discard the supernatant and wash the solid three times with 5 mL portions of water, wash the solid with 3 mL MeOH, dry under reduced pressure, elute with 3 mL 4 M acetic acid. Evaporate the eluate to dryness under reduced pressure, reconstitute the residue with 90 μL water, inject a 10 μL aliquot. Urine. 5 mL Urine + 5.3 mL 2 M HCl, heat at 100° for 20 min, cool to room temperature, add 1 mL 50 mM disodium EDTA, adjust the pH to 8.5 with dilute ammonia, add 500 mg 200 mesh aluminum oxide (Wako), shake for 10 min, filter, wash the solid with 10 mL water, elute with 5 mL 300 mM acetic acid, inject an aliquot of the eluate.

HPLC VARIABLES
Column: 250 × 3.6 10-25 μm Hitachi 3011 C resin
Mobile phase: 50 mM K_2HPO_4 containing 0.05% phosphoric acid
Column temperature: 45
Flow rate: 0.6
Injection volume: 10
Detector: F ex 383 em 486 following post-column reaction. The column effluent mixed with 1% 2-cyanoacetamide in water pumped at 0.5 mL/min and with buffer pumped at 1 mL/min and the mixture flowed through a 5 m × 0.5 mm ID PTFE coil at 100 ± 1° to the detector. (Buffer was 600 mM boric acid containing 750 mM KOH.)

CHROMATOGRAM
Retention time: 6
Limit of detection: 0.11 pmole

OTHER SUBSTANCES
Extracted: dopamine, epinephrine

KEY WORDS
post-column reaction; serum; SPE

REFERENCE
Honda,S.; Takahashi,M.; Araki,Y.; Kakehi,K. Postcolumn derivatization of catecholamines with 2-cyanoacetamide for fluorimetric monitoring in high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *274*, 45–52.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a 150 μL Toyopak IC-SP S (sulfopropyl resin, H^+ form) SPE cartridge (Tosoh) with 10 mL water. Plasma. 700 μL Plasma + 50 μL 700 nM 3,4-dihydroxybenzylamine + 350 μL 2 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and add it to 30 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min. Add a 500 μL aliquot of the supernatant to the SPE cartridge, wash with 1 mL water, wash with 500 μL EtOH:water 50:50, wash with 5 mL water, elute with 500 μL 2 M sodium perchlorate, filter (0.2 μm), inject a 50 μL aliquot of the filtrate. Urine. Acidify urine collected over 24 h with 10 mL 6 M HCl. 500 μL Urine + 25 μL 10 μM 3,4-dihydroxybenzylamine + 25 μL 40 μM ferulic acid + 500 μL 1 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and add it to 30 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min, add a 500 μL aliquot of the supernatant to the SPE cartridge, wash with 1.5 mL water, wash with 500 μL EtOH:water 50:50,

wash with 5 mL water, elute with 500 μL 2 M sodium perchlorate, filter (0.2 μm), inject a 50 μL aliquot of the filtrate.

HPLC VARIABLES

Column: 150 × 4.6 5 μm TSK-gel ODS-80TM (Tosoh)
Mobile phase: Gradient. A was buffer. B was MeCN:MeOH:buffer 8:12:80, pH 3.1. A:B 100:0 for 4 min, to 60:40 over 8 min, to 0:100 over 2 min, maintain at 0:100 for 16 min, return to initial conditions (step gradient), re-equilibrate for 20 min. Buffer was 60 mM pH 3.1 citric acid containing 32 mM Na_2HPO_4, 1.7 mM sodium hexanesulfonate, and 0.1 mM disodium EDTA (J. Chromatogr. 1989, 467, 237).
Flow rate: 1
Injection volume: 50
Detector: F ex 345 em 480 following post-column reaction. The column effluent passed through a Hitachi 655A electrochemical detector with carbon cloth electrodes; working electrode at +0.68 V versus reference electrode (200 mM equimolar mixture of potassium hexacyanoferrate(II) and potassium hexacyanoferrate(III) containing 200 mM potassium nitrate and 200 mM KOH). The effluent from the electrochemical detector mixed with 20 mM meso-1,2-diphenylethylenediamine in 50 mM HCl pumped at 0.4 mL/min and with 1 M glycine containing 490 mM KOH and 3 mM potassium hexacyanoferrate(III) pumped at 0.4 mL/min. This mixture flowed through a 10 m × 0.47 mm ID coil at 80° to the detector (J. Chromatogr. 1989, 467, 237).

CHROMATOGRAM

Retention time: 6
Internal standard: 3,4-dihydroxybenzylamine (12.5)
Limit of detection: 0.6 nM

OTHER SUBSTANCES

Extracted: dopamine, epinephrine, levodopa, metanephrine, 3-methoxytyramine

KEY WORDS

post-column reaction; plasma; SPE

REFERENCE

Nohta,H.; Yamaguchi,E.; Ohkura,Y.; Watanabe,H. Measurement of catecholamines, their precursor and metabolites in human urine and plasma by solid-phase extraction followed by high-performance liquid chromatography with fluorescence derivatization, *J.Chromatogr.*, **1989**, *493*, 15–26.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 3 mL ice-cold MeOH:500 mM perchloric acid 98:2, centrifuge at 4° at 4000 g for 3 min. Remove 200 μL of the supernatant and add it to 100 μL 80 ng/mL N-methyldopamine, evaporate to dryness under vacuum, reconstitute in 200 μL mobile phase, inject a 5-20 μL aliquot. Urine. 1 mL Urine + 50 mL water, inject a 10 μL aliquot. (To deconjugate adjust pH to 1, flush with nitrogen, heat in a boiling water bath for 1 h, dilute with 50 mL water, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeOH:13 mM sodium acetate containing 0.5 mM sodium 1-octanesulfonate and 0.5 mM disodium EDTA 14:86, pH 3.10
Flow rate: 1
Injection volume: 5-20
Detector: E, ESA Model 5100 A Coulochem, Model 5011 A analytical cell, first electrode +0.40 V, second electrode -0.30 V

CHROMATOGRAM

Retention time: 5
Internal standard: N-methyldopamine (11)

OTHER SUBSTANCES

Extracted: methyldopa, epinephrine, dopamine, dihydroxyphenylacetic acid, 3-O-methylmethyldopa, homovanilic acid

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lucarelli,C.; Betto,P.; Ricciarello,G.; Grossi,G. High-performance liquid chromatographic determination of L-3-(3,4-dihydroxyphenyl)-2-methylalanine (α-methyldopa) in human urine and plasma, *J.Chromatogr.*, **1991**, *541*, 285–296.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Toyopak IC-SP S sulfopropyl resin, H^+ form, SPE cartridge (Tosoh) with 10 mL water and 2 mL 200 mM pH 5.0 sodium phosphate buffer. Plasma. 700 μL Plasma + 30 μL 700 nM isoproterenol + 50 μL 7 μM 3,4-dihydroxyphenylpropanoic acid + 350 μL 2 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and adjust the pH to 1.5-2.0 with about 150 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min, add the supernatant to the SPE cartridge, wash with 10 mL water, elute with 300 μL MeOH:2 M sodium perchlorate 7:93, filter (cellulose acetate membrane), inject a 100 μL aliquot of the filtrate. Urine. Collect human urine for 24 h in the presence of 10 mL 6 M HCl. 500 μL Urine + 10 μL 15 μM isoproterenol + 25 μL 800 μM 3,4-dihydroxyphenylpropanoic acid + 500 μL 1 M perchloric acid, mix, centrifuge at 4° at 1000 g for 15 min. Remove a 700 μL aliquot of the supernatant and adjust the pH to 1.5-2.0 with about 130 μL 2 M potassium carbonate, centrifuge at 4° at 1000 g for 5 min, add the supernatant to the SPE cartridge, wash with 1.5 mL water, wash with 500 μL EtOH:water 50:50, wash with 5 mL water, elute with 500 μL 1.5 M KCl in MeOH:100 mM HCl 7:93, filter (cellulose acetate membrane), inject a 100 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm TSK-gel ODS-80TM (Tosoh)
Mobile phase: MeOH:buffer 7:93 (Buffer was 30 mM pH 2.5 citrate buffer containing 0.4 mM sodium octanesulfonate.)
Flow rate: 0.8
Injection volume: 100
Detector: F ex 350 em 480 following post-column reaction. The column effluent mixed with reagent A pumped at 0.3 mL/min and the mixture flowed through a 3 m × 0.5 mm ID stainless steel coil at 90°. The effluent from this coil mixed with reagent B pumped at 0.3 mL/min and the mixture flowed through a 10 m × 0.5 mm ID stainless steel coil at 90° and through a 1 m × 0.5 mm ID stainless steel cooling coil to the detector (Anal. Sci. 1991, 7, 257). (Reagent A was 10 mM sodium periodate containing 3 mM potassium ferricyanide. Reagent B was 30 mM meso-1,2-diphenylethylenediamine in EtOH:water 70:30 containing 130 mM sodium methylate.)

CHROMATOGRAM
Retention time: 16
Internal standard: isoproterenol (60)
Limit of detection: 0.4-0.5 nM

OTHER SUBSTANCES
Extracted: dopamine, epinephrine, levodopa, metanephrine, 3-methoxytyramine, normetanephrine

KEY WORDS
post-column reaction; plasma; SPE

REFERENCE
Jeon,H.-K.; Nohta,H.; Ohkura,Y. High-performance liquid chromatographic determination of catecholamines and their precursor and metabolites in human urine and plasma by postcolumn derivatization involving chemical oxidation followed by fluorescence reaction, *Anal.Biochem.*, **1992**, *200*, 332–338.

SAMPLE
Matrix: blood, urine
Sample preparation: 2 mL Plasma or 1 mL urine + dihydroxybenzylamine + 20 mg Sigma WA4 alumina + 200 μL 1 M pH 8.6 Tris-EDTA buffer, mix for 10 min, discard plasma. Wash the

alumina three times with 3 mL water and dry it. Add 125 μL 500 mM phosphoric acid, after 1 min inject a 100 μL aliquot. (Ann. Clin. Biochem. 1985, 22, 194-203)

HPLC VARIABLES
Column: 250 × 4.5 5 μm Ultratechsphere
Mobile phase: Per liter 75 mmol citric acid, 58.5 mmol NaH_2PO_4, 0.2 mmol disodium EDTA, and 4.4 mmol heptanesulfonic acid, pH adjusted to 3.4, made up to a final volume of 2 L, add 200 mL MeOH
Flow rate: 1
Injection volume: 100
Detector: E, ESA Coulochem conditioning cell +0.35 V, first electrode +0.05 V, second electrode -0.35 V

CHROMATOGRAM
Retention time: 6.00
Internal standard: dihydroxybenzylamine (10.53)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: levodopa, metanephrine, epinephrine, 3-methoxytyrosine, normetanephrine, dihydroxyphenylacetic acid, dopamine

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KEY WORDS
plasma

REFERENCE
Dutton,J.; Copeland,L.G.; Playfer,J.R.; Roberts,N.B. Measuring L-dopa in plasma and urine to monitor therapy of elderly patients with Parkinson disease treated with L-dopa and a dopa decarboxylase inhibitor, *Clin.Chem.*, **1993**, *39*, 629–634.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 2.8

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: cell cultures
Sample preparation: Centrifuge at 20000 g for 5 min, dilute the supernatant 10-50-fold, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 125 × 3 3 μm Nucleosil 100C18
Mobile phase: MeOH:buffer 5:95, pH adjusted to 3.0 with 5 M NaOH (Buffer was 50 mM citric acid containing 30 mM phosphoric acid, 0.75 mM octylsulfate, and 0.5 mM EDTA.)
Column temperature: 40
Flow rate: 0.7
Injection volume: 30
Detector: E, Waters 460, glassy carbon electrode

CHROMATOGRAM
Limit of detection: 10 nM

OTHER SUBSTANCES
Extracted: epinephrine

REFERENCE
Ghindilis,A.L.; Michael,N.; Makower,A. A new sensitive and simple method for detection of catecholamines from adrenal chromaffin cells, *Pharmazie*, **1995**, *50*, 599–600.

SAMPLE
Matrix: dialysate
Sample preparation: Mix 30 μL dialysate + 5 μL 200 mM perchloric acid, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 80 × 4.6 3 μm HR-80 C18 (ESA)
Mobile phase: MeCN:MeOH:buffer 15:13:72 (Buffer was 75 mM NaH_2PO_4, 1.5 mM sodium dodecyl sulfate, 100 μL/L triethylamine, and 20 μm EDTA, adjusted to pH 5.6.)
Column temperature: 25
Flow rate: 1
Injection volume: 20
Detector: E, ESA Coulochem II, Model 5014 Microdialysis Cell with E1 -175 mV and E2 +175 mV, Pd reference electrode, Model 5020 Guard Cell EGC +300 mV

CHROMATOGRAM
Retention time: 2.83
Limit of detection: 400 fg

OTHER SUBSTANCES
Extracted: dopamine, epinephrine, serotonin

KEY WORDS
rat; brain; pharmacokinetics; use PEEK tubing and sample loop

REFERENCE
Gariepy,K.C.; Bailey,B.; Yu,J.; Maher,T.; Acworth,I.N. Simultaneous determination of norepinephrine, dopamine, and serotonin in hippocampal microdialysis samples using normal bore high performance liquid chromatography: Effects of dopamine receptor agonist stimulation and euthanasia, *J.Liq.Chromatogr.*, **1994**, *17*, 1541–1556.

SAMPLE
Matrix: dialysate
Sample preparation: 20 μL Dialysate (Ringer's solution) + 50 μL 80 pg/mL 3,4-dihydroxybenzylamine in 2% acetic acid + 5 mg acid-washed alumina + 1 mL 1 M pH 8.6 Tris buffer containing 0.2% disodium EDTA, shake for 15 min, wash the alumina 3 times with water. Place

the alumina in an Ultrafree C3 microfilter tube (Millipore), dry by centrifuging at 600 g for 5 min, elute with 60 μL 2% acetic acid, inject a 50 μL aliquot of the eluate.

HPLC VARIABLES
Guard column: 5 × 4 AC-ODS (Eicom)
Column: 150 × 2.1 Eicompak CA-50DS (Eicom)
Mobile phase: MeOH:100 mM pH 6.1 phosphate buffer 10:90 containing 600 μg/mL sodium 1-octanesulfonate
Column temperature: 25
Flow rate: 0.25
Injection volume: 50
Detector: E, Eicom ECD-300, graphite electrode + 400 mV, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 7
Internal standard: 3,4-dihydroxybenzylamine (14)
Limit of detection: 5 pg/mL

KEY WORDS
SPE; cat

REFERENCE
Yamazaki,T.; Akiyama,T.; Shindo,T. Routine high-performance liquid chromatographic determination of myocardial interstitial norepinephrine, *J.Chromatogr.B*, **1995**, *670*, 328–331.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Dissolve powdered tablets in 10 mM HCl, filter if necessary, inject an aliquot. Injections, solutions. Dilute with 10 mM HCl, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Partisil-5 ODS-3
Mobile phase: MeOH:buffer 30:70 (Buffer was 10 mM sodium 1-octanesulfonate in 0.2% acetic acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 10.5
Limit of detection: 27 ng

OTHER SUBSTANCES
Simultaneous: epinephrine, levonordefrin, isoproterenol, phenylephrine, metaraminol, impurities

KEY WORDS
tablets; injections; ophthalmic solutions; inhalation solutions

REFERENCE
Smela,M.J.,Jr.; Stromberg,R. Liquid chromatographic determination of six sympathomimetic drugs in dosage forms, *J.Assoc.Off.Anal.Chem.*, **1991**, *74*, 289–291.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Grind tablets, weigh out a portion, dissolve in 50 mL mobile phase, sonicate, filter (No. 4 sintered glass plate), dilute, inject an aliquot. Capsules. Dissolve 10 capsules (without opening) in 100 mL mobile phase, sonicate, inject an aliquot. Injections, ampules, sprays. Dilute, inject an aliquot.

HPLC VARIABLES
Column: 120 × 4.6 Spherisorb C18 ODS-2
Mobile phase: Isopropanol:buffer 5:95 (Buffer was 100 mM sodium dodecyl sulfate containing 25 mM Na_2HPO_4, pH adjusted to 3.0 with HCl.)
Flow rate: 1
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: k' 3.1
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Simultaneous: carbidopa, dopamine, epinephrine, hydrochlorothiazide, isoproterenol, levodopa, methyldopa, phenylephrine

KEY WORDS
tablets; capsules; injections; ampules; sprays

REFERENCE
Villanueva Camañas,R.M.; Sanchis Mallols,J.M.; Torres Lapasió,J.R.; Ramis-Ramos,G. Analysis of pharmaceutical preparations containing catecholamines by micellar liquid chromatography with spectrophotometric detection, *Analyst*, **1995**, *120*, 1767–1772.

SAMPLE
Matrix: perfusate
Sample preparation: 30 μL Perfusate (artificial CSF) + 10 μL 200 mM perchloric acid. Mix a 25 μL aliquot with 12.5 μL reagent, let stand for 2 min, inject an aliquot. (Prepare a stock solution by dissolving 27 mg o-phthalaldehyde in 1 mL MeOH, add 5 μL β-mercaptoethanol, add 9 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA. This solution is good for 5 days in a sealed amber bottle at room temperature. Prepare the working reagent by diluting 1 mL of the stock solution with 3 mL 100 mM pH 9.3 sodium tetraborate containing 10 μM EDTA, allow to stand for 24 h before use.)

HPLC VARIABLES
Column: two columns 150 × 4.6 5 μm M.S. Gel C18 (ESA)
Mobile phase: MeOH:buffer 8:92 adjusted to pH 3.0 with phosphoric acid (Buffer was 54 mM NaH_2PO_4 containing 1.24 mM sodium heptanesulfonate.)
Column temperature: 33
Flow rate: 1.2
Detector: E, ESA Coulochem Electrode Array System Model 5500, detector temp 33°, oxidation potential 70 mV

CHROMATOGRAM
Retention time: 2.51
Limit of quantitation: 0.5 ng/mL

OTHER SUBSTANCES
Extracted: apomorphine, dopamine, hydralazine, isoproterenol, methoxamine, morphine, phenylephrine

KEY WORDS
rat; derivatization

REFERENCE
Acworth,I.N.; Yu,J.; Ryan,E.; Gariepy,K.C.; Gamache,P.; Hull,K.; Maher,T. Simultaneous measurement of monoamine, amino acid, and drug levels, using high performance liquid chromatography and coulometric array technology: application to in vivo microdialysis perfusate analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 685–705.

SAMPLE
Matrix: perfusate
Sample preparation: Perfusate + 20 μL 25 pM dihydroxybenzylamine + 15 mg activated alumina + pH 8.3-8.5 Tris buffer containing 1% EDTA, mix. Remove the alumina and wash it with water, add 100 μL 200 mM perchloric acid, centrifuge, store supernatant on ice, inject a 20 μL aliquot.

HPLC VARIABLES
Column: Lichrospher 60 RP-select B C18
Mobile phase: MeCN:buffer 1.5:98.5 (Buffer was 100 mM NaH_2PO_4 containing 1.4 mM sodium 1-octanesulfonate and 0.2 mM EDTA, pH 2.5.)
Flow rate: 0.8
Injection volume: 20
Detector: E, BAS LC3A, 0.8 V

CHROMATOGRAM
Retention time: 10
Internal standard: dihydroxybenzylamine (16)
Limit of detection: 20 fmole

KEY WORDS
SPE

REFERENCE
Forray,M.I.; Andrés,M.E.; Bustos,G.; Gysling,K. Regulation of endogenous noradrenaline release from the bed nucleus of stria terminalis, *Biochem.Pharmacol.*, **1995**, *49*, 687–692.

SAMPLE
Matrix: solutions
Sample preparation: Dilute a few μL of a <1 mM solution to 20 μL with 50 mM pH 8.0 phosphate or borate buffer, add 10 μL 2 mg/mL (?) fluorescamine in acetone with vigorous shaking, inject an aliquot.

HPLC VARIABLES
Column: 500 × 3 Hitachi 3011 gel glass column
Mobile phase: MeOH:100 mM pH 8.0 Tris-HCl buffer 70:30
Flow rate: 0.72
Detector: F primary filter Corning No. 7-51, secondary filter No. 4-7116

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: dopamine
Also analyzed: 3-methoxytyramine, normetanephrine

KEY WORDS
derivatization

REFERENCE
Imai,K. Fluorimetric assay of dopamine, norepinephrine and their 3-O-methyl metabolites by using fluorescamine, *J.Chromatogr.*, **1975**, *105*, 135–140.

SAMPLE
Matrix: solutions
Sample preparation: 200 μL 5 μg/mL Amine solution + 300 μL 100 mM pH 8.0 phosphate buffer + 300 μL 200 μg/mL fluorescamine in acetone + 200 μL water, mix, saturate with NaCl, add 500 μL ethyl acetate, shake for 1 min, inject a 50 μL aliquot of the organic phase.

HPLC VARIABLES
Column: 250 × 2 10 μm LiChrosorb Si 60-10

Mobile phase: Benzene:dioxane:acetic acid 76:22:2 (Caution! Benzene and dioxane are carcinogens!)
Flow rate: 0.5
Injection volume: 50
Detector: F ex 325-385 (filter) em 451

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: dopamine

KEY WORDS
derivatization; normal phase

REFERENCE
Schwedt,G. Hochdruck-Flüssigkeits-chromatographische Analyse der Katecholamine Dopamin und Noradrenalin als Fluorescaminderivate, *J.Chromatogr.*, **1976**, *118*, 429–432.

SAMPLE
Matrix: solutions
Sample preparation: Dilute with 5% dextrose, inject a 15 μL aliquot.

HPLC VARIABLES
Column: Waters microparticulate C18
Mobile phase: MeOH:350 mM acetic acid and 5 mM sodium heptanesulfonate 15:85
Flow rate: 1.6-2.0
Injection volume: 15
Detector: F ex 285 em 315

CHROMATOGRAM
Retention time: 4.75

OTHER SUBSTANCES
Interfering: dopamine

REFERENCE
Williams,D.A.; Fung,E.Y.Y.; Newton,D.W. Ion-pair high-performance liquid chromatography of terbutaline and catecholamines with aminophylline in intravenous solutions, *J.Pharm.Sci.*, **1982**, *71*, 956–958.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 250 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 5-C18
Mobile phase: 50 mM Potassium perchlorate containing 250 μL/L 3% copper acetate in water and 10 g/L sodium acetate, pH adjusted to 4.45 with acetic acid
Flow rate: 1
Injection volume: 250
Detector: F ex 400 em 500 following post-column reaction. The column effluent flowed through the reactor then through a 3.5 m × 0.8 mm ID coil of PTFE tubing at 30°. The effluent from the coil mixed with reducing solution pumped at 1 (?) mL/min and this mixture flowed through a 2 m × 0.8 mm ID PTFE coil at 30° to the detector. (Prepare the reactor as follows. Dissolve 75.3 g manganese nitrate in 500 mL water, add 50 g 18-35 mesh silica gel (Macherey-Nagel), stir vigorously, slowly add 31.6 g potassium permanganate in 500 mL water, stir for 30 min, filter (500 μm sieve), wash until no permanganate color is left, dry in a desiccator, pack in a 50 × 2.1 stainless steel tube. The reducing solution was 266 g NaOH, 13.4 g anhydrous sodium sulfite, and 9 mL 2-mercaptoethanol in 1 L water. Note that some Nucleosil 5-C18 column packings do not give separation at pH 4.45. In this case it is necessary to use 50 mM perchloric

acid as mobile phase and mix the column effluent with pH 4.4 sodium acetate buffer before it enters the reactor.)

CHROMATOGRAM
Retention time: 7
Limit of detection: 1 ng/mL

OTHER SUBSTANCES
Extracted: epinephrine

KEY WORDS
post-column reaction

REFERENCE
Rüter,J.; Kurz,U.P.; Neidhart,B. Solid phase reactors as an analytical tool in the determination of urinary noradrenaline and adrenaline, *J.Liq.Chromatogr.*, **1985**, *8*, 2475–2496.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid,

progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 4.90

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 150 × 4 5 μm Crownpak CR(+) immobilized crown ether
Mobile phase: 0.1% pH 1.9 Perchloric acid
Column temperature: 25
Flow rate: 1
Detector: UV 210

CHROMATOGRAM
Retention time: 3.88, 4.18

OTHER SUBSTANCES
Simultaneous: octopamine

KEY WORDS
chiral; comparison with capillary electrophoresis

REFERENCE
Nishi,H.; Nakamura,K.; Nakai,H.; Sato,T. Separation of enantiomers and isomers of amino compounds by capillary electrophoresis and high-performance liquid chromatography utilizing crown ethers, *J.Chromatogr.A*, **1997**, *757*, 225–235.

SAMPLE
Matrix: tissue
Sample preparation: Prepare a 70 × 5 SPE column of Sephadex G 10 in a Pasteur pipette, wash with 3 mL 20 mM ammonia and 3 mL 10 mM formic acid. Homogenize up to 150 mg rat brain in 1 mL 100 mM perchloric acid, centrifuge at 4000 g at 4° for 15 min, add 500 μL of the supernatant to the SPE column, wash with 2.5 mL 10 mM formic acid, elute with 1 mL 10 mM formic acid followed by 1.5 mL 5 mM Na_2HPO_4, inject an aliquot of the eluate.

HPLC VARIABLES
Column: Nucleosil 5 C18
Mobile phase: pH 6.5 Buffer prepared from 200 mM Na_2HPO_4 and 100 mM citric acid
Flow rate: 0.7
Injection volume: 200
Detector: E, rotating disc electrode, 500 mV

CHROMATOGRAM
Retention time: 4
Limit of detection: 0.05 nmole/g

OTHER SUBSTANCES
Extracted: uric acid

KEY WORDS
rat; brain; SPE

REFERENCE
Westerink,B.H.C.; Mulder,T.B.A. Determination of picomole amounts of dopamine, noradrenaline, 3,4-dihydroxyphenylacetic acid, homovanillic acid, and 5-hydroxyindolacetic acid in nervous tissue after one-step purification on Sephadex G-10, using high-performance liquid chromatography with a novel type of electrochemical detection, *J.Neurochem.*, **1981**, *36*, 1449–1462.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (glass potters) with 5 volumes of 100 mM perchloric acid containing 1.9 mM sodium bisulfite, centrifuge at 10000 g at 4° for 30 min. Filter (0.22 μm) the supernatant, add dihydroxybenzylamine, inject a 5-20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 Hypersil H5 ODS
Mobile phase: EtOH:buffer 2:98 (Buffer was 13.8 g NaH_2PO_4, 60 mg disodium EDTA, and 20 mg 1-octanesulfonic acid in 1 L water, pH adjusted to 3.70 with phosphoric acid.)
Flow rate: 1
Injection volume: 5-20
Detector: E, Unicam PU 4022, 70 mV

CHROMATOGRAM
Retention time: 5
Internal standard: dihydroxybenzylamine

OTHER SUBSTANCES
Extracted: dopamine, epinephrine

KEY WORDS
adrenal; fetal

REFERENCE
Garciá,J.C.; Blanco,L.; McPherson,M.; Leiva,A.; Maciás,R. High-performance liquid chromatographic determination of norepinephrine, epinephrine and dopamine in human foetal adrenal gland, *J.Chromatogr.B*, **1994**, *656*, 77–80.

SAMPLE
Matrix: urine
Sample preparation: Acidify urine with 1% (v/v) 6 M HCl. 6-10 mL Swine urine or 1-2.5 mL rat urine, centrifuge at 4000 g for 30 min, add 200 ng 3,4-dihydroxybenzylamine hydrobromide and 15 mL 1g/L EDTA, adjust to pH 6.45-6.55 with HCl or NaOH. Add the mixture to a cation-exchange resin SPE cartridge (Bio-Rad), wash twice with 10 mL water and with 5 ml water, elute with 8 mL 10 g/L boric acid. Dilute boric acid eluate with an equal volume of mobile phase, inject a 60 μL aliquot. (Procedure for determining methoxycatecholamines is also described.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Kromasil C8
Mobile phase: MeOH:buffer 15:85 (Mobile phase was 300 mL MeOH, 1.5 mL 200 mg/mL 1-octanesulfonic acid, 100 mL 1 M sodium acetate, and about 1 L water. The pH was adjusted to pH 3.8 with citric acid and made up to 2 L with water.)
Flow rate: 0.6
Injection volume: 60
Detector: E, Bioanalytical Systems, glassy carbon electrode + 650 mV, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 7.28
Internal standard: 3,4-dihydroxybenzylamine hydrobromide (10.31)
Limit of detection: 40 pg

OTHER SUBSTANCES
Extracted: dopamine, epinephrine

KEY WORDS
pig; rat; SPE; pharmacokinetics

REFERENCE
Hay,M.; Mormède,P. Determination of catecholamines and methoxycatecholamines excretion patterns in pig and rat urine by ion-exchange liquid chromatography with electrochemical detection, *J.Chromatogr.B*, **1997**, *703*, 15–23.

SAMPLE
Matrix: urine
Sample preparation: Acidify urine by adding 1% 6 M HCl. 5 mL Acidified urine + 1 mL 7.5% disodium EDTA, adjust pH to 8.5 with 1 M NaOH, add 250 mg alumina (previously treated with 2 M HCl), shake for 5 min, decant the supernatant, wash the alumina three times with 5 mL portions of water. Place the alumina in a 4 mm ID glass column, elute with 250 mM acetic acid in water, collect 2.5 mL eluate, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 1000 × 2.1 Zipax SCX (DuPont)
Mobile phase: MeCN:50 mM NaH_2PO_4 5:95
Column temperature: 40
Flow rate: 0.8
Injection volume: 100
Detector: F ex 400 em 490 following post-column reaction. The column effluent mixed with the reagent pumped at 0.4 mL/min and the mixture flowed through a 10 m × 0.5 mm PTFE coil at 75 ± 0.1° to the detector. (Reagent was 500 mM borate buffer adjusted to pH 9.7 with NaOH.)

CHROMATOGRAM
Retention time: 5.5
Limit of quantitation: 0.25 ng

OTHER SUBSTANCES
Extracted: epinephrine

KEY WORDS
post-column reaction; SPE

REFERENCE
Nimura,N.; Ishida,K.; Kinoshita,T. Novel post-column derivatization method for the fluorimetric determination of norepinephrine and epinephrine, *J.Chromatogr.*, **1980**, *221*, 249–255.

SAMPLE
Matrix: urine
Sample preparation: 1 mL Urine + 2 mL water + 1 mL 500 μM EDTA in 1 mM HCl + 1 mL 2 M pH 8.5 phosphate buffer + 20 μL 100 μM 3,4-dihydroxybenzylamine + 50 mg alumina, vortex for 3 min, discard the supernatant, wash the alumina 3 times with 5 mL portions of water, elute by washing the alumina twice with 200 μL portions of 100 mM phosphoric acid for 30 s each time. Combine the acidic layers and add them to 560 μL 400 mM pH 9.0 borate buffer, add 20 μL 50 mM NaCN, add 20 μL 5 mM naphthalene-2,3-carboxaldehyde in MeOH, mix thoroughly, let stand at room temperature for 20 min, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm ODS-120T (Toyo Soda)
Mobile phase: MeCN:THF:10 mM pH 2.5 phosphate buffer 38:6:56
Flow rate: 1
Injection volume: 5
Detector: F ex 420 em 483

CHROMATOGRAM
Retention time: 18
Internal standard: 3,4-dihydroxybenzylamine (15.5)
Limit of detection: 20 fmole

OTHER SUBSTANCES
Extracted: dopamine

KEY WORDS
derivatization; SPE

REFERENCE
Kawasaki,T.; Higuchi,T.; Imai,K.; Wong,O.S. Determination of dopamine, norepinephrine, and related trace amines by prechromatographic derivatization with naphthalene-2,3-dicarboxaldehyde, *Anal.Biochem.*, **1989**, *180*, 279–285.

SAMPLE
Matrix: urine
Sample preparation: Add disodium EDTA and sodium metabisulfite to urine. 100 μL Urine + 2 mL water, vortex, add 1 mL reagent 1, add 5 mL reagent 2, shake vigorously for 2 min, centrifuge at 2000 g for 2 min, freeze in dry ice/acetone. Remove the organic layer and add it to 200 μL 80 mM acetic acid and 2 mL n-octanol saturated with acetic acid, shake vigorously for 2 min, centrifuge at 2000 g for 2 min, freeze in dry ice/acetone until the aqueous layer is just solid, remove the organic layer. Thaw out the aqueous layer and add it to 1 mL reagent 1 and 5 mL reagent 2, shake vigorously for 2 min, centrifuge at 2000 g for 2 min, freeze in dry ice/acetone. Remove the organic layer and add it to 200 μL 80 mM acetic acid and 2 mL n-octanol saturated with acetic acid, shake vigorously for 2 min, centrifuge at 2000 g for 2 min, freeze in dry ice/acetone until the aqueous layer is just solid, remove the organic layer. Thaw out the aqueous layer and add 100 μL Bicine buffer, 250 μL MeCN, and 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, vortex, add 20 μL 20 mM potassium ferricyanide, vortex, heat at 37° for 40 min, cool to room temperature, inject a 100 μL aliquot. (Prepare reagent 1 by dissolving 214 g ammonium chloride and 10 g disodium EDTA in 2 L water, adjust pH to 8.3-8.5 with concentrated ammonium hydroxide, add 4.0 g diphenylborate-ethanolamine complex, stir for several hours until a clear solution is obtained. Prepare reagent 2 by dissolving 2.5 g tetraoctylammonium bromide and 10 mL n-octanol (saturated with acetic acid) in 1 L n-heptane. Prepare Bicine buffer by dissolving 14.3 g Bicine (N,N-bis(2-hydroxyethyl)glycine) and 359 mg anhydrous sodium acetate in 45 mL water, stir overnight until dissolved, adjust pH to 7.30 with concentrated NaOH, make up to 50 mL with water. Note that concentration of 1,2-diphenylethylenediamine is not given in paper. Other authors have used 100 mM (J.Chromatogr. 1989, 487, 17; 1992, 574, 109; 1992, 583, 236).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 40:10:50. B was MeCN:MeOH:50 mM pH 7.0 sodium acetate buffer 50:10:40. A:B 75:25 for 1 min, to 10:90 over 7 min, return to initial conditions over 1 min.
Flow rate: 1
Injection volume: 100
Detector: F ex 365 em 418 (cutoff filter)

CHROMATOGRAM
Retention time: 3
Limit of detection: <0.4 nM

OTHER SUBSTANCES
Extracted: dopamine, epinephrine
Simultaneous: isoproterenol

KEY WORDS
derivatization; protect from light

REFERENCE
Moleman,P.; van Dijk,J. Determination of urinary norepinephrine and epinephrine by liquid chromatography with fluorescence detection and pre-column derivatization, *Clin.Chem.*, **1990**, *36*, 732–736.

SAMPLE
Matrix: urine

Sample preparation: Add 10-15 mL 6 M HCl to a 24 h volume of urine. 2 mL 3 M Tris buffer containing 30 mM EDTA + 500 μL 10 μM dihydrobenzylamide in 100 mM perchloric acid + 2 mL 3 M tris buffer containing 30 mM EDTA + 100 μL 5 M NaOH + 4 mL acidified urine, mix, add to a 1 mL SPE column containing 200 mg alumina (70-230 mesh-ASTM (Touzart et Matignon) at 3 mL/min, wash with 9 mL water at 12 mL/min, force through 0.5 mL air, elute with 1 mL 150 mM perchloric acid at 0.75 mL/min, mix eluate, inject a 100 μL aliquot. (The pH of the Tris buffer is such that the pH of the mixture applied to the SPE column is 7.75-8.0.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil 100/C18
Mobile phase: MeOH:buffer 36:64 (Buffer was 75 mM NaH_2PO_4, 0.15 mM EDTA, and 6 mM sodium heptanesulfonate, pH 3.96.)
Flow rate: 1.25
Injection volume: 100
Detector: F ex 280 em 310

CHROMATOGRAM
Retention time: 6.7
Internal standard: dihydrobenzylamide hydrobromide (9.6)
Limit of detection: 10 nM

OTHER SUBSTANCES
Extracted: dopamine, epinephrine
Simultaneous: levodopa, methyldopa

KEY WORDS
SPE

REFERENCE
Said,R.; Robinet,D.; Barbier,C.; Sartre,J.; Huguet,C. Fully automated high-performance liquid chromatographic assay for the analysis of free catecholamines in urine, *J.Chromatogr.*, **1990**, *530*, 11–18.

SAMPLE
Matrix: urine
Sample preparation: 100 μL Urine + 125 μL 218.6 nM α-methylnorepinephrine in 10 mM HCl + 1 mL 10 mM HCl + 1 mL reagent + 5 mL 4.6 mM tetraoctylammonium bromide in n-heptane: 1-octanol 99:1, shake for 2 min, centrifuge at 20° at 1000 g for 5 min, freeze in dry ice/acetone. Remove the organic phase and add it to 2 mL 1-octanol saturated with 80 mM acetic acid and 200 μL 80 mM acetic acid, shake, centrifuge at 20° at 1000 g for 5 min, freeze in dry ice/acetone. Discard the organic layer and add 1 mL 10 mM HCl to the aqueous layer, add 2 mL 1-octanol saturated with 80 mM acetic acid, add 150 μL 80 mM acetic acid, shake, centrifuge at 20° at 1000 g for 5 min, freeze in dry ice/acetone. Discard the organic layer and add 200 μL MeCN and 50 μL buffer to the aqueous layer, add 100 μL 100 mM 1,2-diphenylethylenediamine in 100 mM HCl, add 20 μL 20 mM potassium ferricyanide in water, heat at 37° in the dark for 1 h, inject a 50 μL aliquot. (Reagent was 8.9 mM diphenylborate-ethanolamine complex in 2 M pH 8.6 ammonia/ammonium chloride buffer containing 13.4 mM EDTA. Buffer was 1.75 M pH 7.05 bicine in water containing 1% EDTA. Recrystallize 1,2-diphenylethylenediamine from toluene:light petroleum (bp 60-80°) 10:90, dry overnight at 60°.)

HPLC VARIABLES
Column: 100 × 4.6 3 μm Cp MicroSpher C18 (Chrompack)
Mobile phase: MeCN:MeOH:50 mM pH 7.0 sodium acetate 40:8:50 (At the end of the day flush column with 60 mL MeCN:MeOH:water 70:10:20.)
Flow rate: 1
Injection volume: 50
Detector: F ex 350 em 480

CHROMATOGRAM
Retention time: 2
Internal standard: α-methylnorepinephrine (Janssen, Beerse, Belgium) (3)
Limit of quantitation: 5.3 nM

OTHER SUBSTANCES
Extracted: dopamine, epinephrine

KEY WORDS
derivatization; protect from light

REFERENCE
van der Hoorn,F.A.J.; Boomsma,F.; Man in 't Veld,A.J.; Schalekamp,M.A.D.H. Improved measurement of urinary catecholamines by liquid-liquid extraction, derivatization and high-performance liquid chromatography with fluorimetric detection, *J.Chromatogr.*, **1991**, *563*, 348–355.

SAMPLE
Matrix: urine
Sample preparation: Condition a 100 mg Bakerbond C-18 SPE cartridge with 2 mL MeOH and 2 mL buffer I. Heat urine at 50°, centrifuge, remove a 500 μL aliquot and add it to 1 mL buffer II (?), add 20 μL 860 ng/mL dihydroxybenzylamine, shake for 5 min, add a 1 mL aliquot to the SPE cartridge, wash with 2 mL buffer I, wash with 1 mL MeOH:buffer I 50:50, wash with 500 μL water, elute with 2 mL 1 M acetic acid, inject a 20 μL aliquot. (Buffer I was 200 mM ammonium chloride containing 0.05% EDTA and 0.4% tetrabutylammonium iodide, pH adjusted to 8.0 ± 0.1. Buffer II was 2 M ammonium chloride containing 0.5% EDTA and 1.2% tetrabutylammonium iodide, pH adjusted to 8.0 ± 0.1.)

HPLC VARIABLES
Column: 150 × 3 5 μm Separon SGX C-18 (Tessek)
Mobile phase: MeOH:buffer 5:95-7:93 (Buffer was 50 mM pH 3.0 ± 0.1 phosphate buffer containing 50 mM EDTA, 1 mM sodium octanesulfonate, and 1 mM NaCl.)
Injection volume: 20
Detector: E, AMOR 400 mV (SunChrom)

CHROMATOGRAM
Internal standard: dihydroxybenzylamine
Limit of detection: 3 ng/mL

OTHER SUBSTANCES
Extracted: dopamine, epinephrine

KEY WORDS
SPE

REFERENCE
Brandsteterova,E.; Krajnak,K.; Skacani,I. HPLC analysis of urinary catecholamines using affinity SPE procedure, *Pharmazie*, **1995**, *50*, 825–826.

SAMPLE
Matrix: urine
Sample preparation: Adjust urine to pH 3.0 with 6 M HCl, centrifuge at 1600 g for 10 min. 900 μL Supernatant + 100 μL 2.5 μM N-methyldopamine, mix, inject a 100 μL aliquot on to column A and elute to waste with mobile phase A, after 5 min elute the contents of column A on to column B with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES
Column: A 35 × 4 TSK-precolumn-CA 2 (Tosoh); B 150 × 4.6 mixed mode (C18/cation exchange) (Alltech)
Mobile phase: A 15 mM pH 6.0 citric acid/trisodium citrate buffer; B 200 mM pH 6.0 citric acid/trisodium citrate buffer
Column temperature: 35
Flow rate: 0.7
Injection volume: 100
Detector: E, Mitsubishi 8 channel, 150 mV

CHROMATOGRAM
Retention time: 10.5
Internal standard: N-methyldopamine (30 mV) (20)
Limit of detection: 1 nM

OTHER SUBSTANCES
Extracted: dopamine (30 mV), epinephrine (150 mV), metanephrine (380 mV), 3-methoxytyramine (340 mV), normetanephrine (380 mV), serotonin (150 mV)

KEY WORDS
column-switching

REFERENCE
Mashige,F.; Matsushima,Y.; Miyata,C.; Yamada,R.; Kanazawa,H.; Sakuma,I.; Takai,N.; Shinozuka,N.; Ohkubo,A.; Nakahara,K. Simultaneous determination of catecholamines, their basic metabolites and serotonin in urine by high-performance liquid chromatography using a mixed-mode column and an eight-channel electrochemical detector, *Biomed.Chromatogr.*, **1995**, *9*, 221–225.

SAMPLE
Matrix: urine
Sample preparation: Condition a 10 × 2 20 mg 15-25 μm PLRP-S polymer-based SPE cartridge (Spark Holland) with 1 mL MeOH, with 0.5 mL water, and with 1.5 mL 200 mM pH 8.5 ammonia/ammonium chloride buffer containing 0.05% EDTA. Collect 24 h urine with 10 mL 6 M HCl, final pH 1-3. Dilute 4-fold with buffer. Inject a 200 μL aliquot onto the SPE cartridge, wash with 1 mL 200 mM pH 8.5 ammonia/ammonium chloride buffer containing 0.05% EDTA, wash with 250 μL MeOH:200 mM pH 8.5 ammonia/ammonium chloride buffer 20:80, wash with water at 1 mL/min for 2.25 min. Elute the contents of the SPE cartridge onto column A with the mobile phase for 30 s then remove the SPE cartridge from the circuit, elute column A with mobile phase onto column B, after 1.25 min elute column A to waste with mobile phase and elute column B with mobile phase, monitor the effluent from column B. (Buffer was 2 M pH 8.5 ammonia/ammonium chloride containing 0.5% EDTA, 0.1% diphenylborate, and 18 ng/mL dihydroxybenzylamine.)

HPLC VARIABLES
Column: A 30 × 4.6 C18 (Brownlee); B 250 × 4.6 5 μm Ultrasphere IP C18
Mobile phase: MeCN:MeOH:buffer 15:8:100, apparent pH adjusted to 3.2 with 1.5 M orthophosphoric acid (Buffer was 50 mM KH_2PO_4 containing 1 mM sodium heptane sulphate and 0.07 mM EDTA.)
Flow rate: 0.8
Injection volume: 200
Detector: E, ESA Model 5100A, Model 5021 conditioning cell, Model 5011 analytical cell, oxidizing electrode +350 mV, screen electrode +100 mV, quantifying electrode -300 mV

CHROMATOGRAM
Retention time: 6
Internal standard: dihydroxybenzylamine (8)
Limit of detection: 1.3 ng/mL

OTHER SUBSTANCES
Extracted: epinephrine, dopamine

KEY WORDS
SPE; column-switching

REFERENCE
Pastoris,A.; Cerutti,L.; Sacco,R.; De Vecchi,L.; Sbaffi,A. Automated analysis of urinary catecholamines by high-performance liquid chromatography and on-line sample pretreatment, *J.Chromatogr.B*, **1995**, *664*, 287–293.

SAMPLE
Matrix: urine

Sample preparation: Acidify urine to pH 2.0-3.5 with 5 M HCl, centrifuge at 7000 g for 10 min, inject a 10-500 μL aliquot on to column A and elute to waste with mobile phase A, after 10 min backflush the contents of column A on to column B with mobile phase B, elute with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A nitrophenylboronic acid modified copolymer (U.S. Patent 4 767 529 (Chem.Abs. 1988, 108, 71698t)); B 53 × 4.6 1.5 μm MICRA NPS RP-18 (MICRA Scientific, Northbrook)
Mobile phase: A 20 mM $(NH_4)_2HPO_4$ containing 10 mM EDTA, adjusted to pH 8.7 with 25% ammonia solution; B 10 mM NaH_2PO_4 containing 0.1 mM dodecanesulfonic acid, adjusted to pH 2.5 with orthophosphoric acid
Flow rate: A 0.5; B from 0.2 to 0.5 over 2 min, maintain at 0.5
Injection volume: 10-500
Detector: F ex 275 em 330

CHROMATOGRAM

Retention time: 4
Limit of detection: 2.43 pmole
Limit of quantitation: 5.57 pmole

OTHER SUBSTANCES

Extracted: dopamine, epinephrine

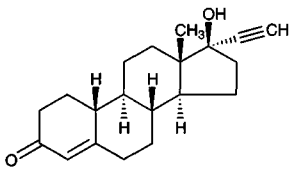
KEY WORDS

column-switching

REFERENCE

Rudolphi,A.; Boos,K.-S.; Seidel,D. Coupled-column HPLC analysis of free urinary catecholamines using restricted access affinity precolumn and micro-particulate nonporous silica analytical column, *Chromatographia*, **1995**, *41*, 645–650.

Norethindrone



Molecular formula: $C_{20}H_{26}O_2$
Molecular weight: 298.43
CAS Registry No.: 68-22-4, 51-98-9 (acetate)
Merck Index: 6790
Lednicer No.: 1 164, 165; 2 145

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30

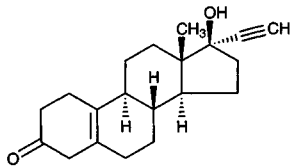
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 240.5

CHROMATOGRAM
Retention time: 24.038

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Norethynodrel



Molecular formula: $C_{20}H_{26}O_2$
Molecular weight: 298.43
CAS Registry No.: 68-23-5
Merck Index: 6791
Lednicer No.: 1 168

SAMPLE
Matrix: solutions

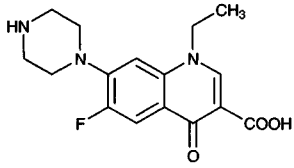
HPLC VARIABLES
Column: 50 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeOH:THF:water 10:20:70
Flow rate: 2
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 6.2 (norethynodrel acetate)

OTHER SUBSTANCES
Simultaneous: ethinyl estradiol, norethindrone, norethindrone acetate, norgestrel

REFERENCE
Supelco Catalog, **1994**, p. 779.

Norfloxacin



Molecular formula: $C_{16}H_{18}FN_3O_3$
Molecular weight: 319.34
CAS Registry No.: 70458-96-7, 100587-52-8 (norfloxacin succinil)
Merck Index: 6793
Lednicer No.: 4 141, 143

SAMPLE
Matrix: aqueous humor, blood, tissue